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(71) Applicant *(for all designated States except US)*: UNIPATH LIMITED [GB/GB]; Wade Road, Basingstoke, Hampshire RG24 0PW (GB).

(71) Applicant *(for US only)*: SAWHNEY, Rohini (heiress of the deceased inventor) [GB/GB]; 38 Petersfield Close, Chineham, Basingstoke, Hampshire RG24 8WP (GB).

(72) Inventor: SAWHNEY, Deepak, Raj (deceased).

(74) Agent: BUTLER, David, John; Unilever plc, Patent Division, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB).

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(54) Title: METHODS AND APPARATUS FOR DETECTING MICROORGANISMS

(57) Abstract

An "electronic nose" comprising an array of different odour-reactive sensors is used to enhance the speed of detection of viable microorganisms in blood culture, by detecting the level of volatile compounds in the atmosphere adjacent a culture medium. An earlier determination of the identity of the proliferating microorganisms can also be derived.

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Methods and Apparatus for Detecting Micro-Organisms

FIELD OF THE INVENTION

This invention relates to methods and apparatus for detecting micro-organisms, for example in blood culture.

BACKGROUND TO THE INVENTION

The screening of biological samples and other materials for the possible presence of contaminating micro-organisms is conducted on a very large scale. A particular example is the screening of body fluid samples, especially blood samples, for the possible presence of pathogenic organisms. In a typical clinical laboratory, hundreds of blood samples are processed daily. Many systems have been proposed for automating and de-skilling such procedures. A variety of commercial systems exist. Although much work has gone into speeding up the technology, the systems available today are still slow. In a typical blood sample analysing system, the individual blood sample is injected into a bottle containing a liquid culture medium. The inoculated bottle is incubated for example over-night. Over a number of hours, viable micro-organisms present in the original sample can metabolise and proliferate in the medium. Eventually this proliferation can lead to a significant change in the content of the gaseous headspace within the bottle. Most commercially-available systems are designed to detect a significant increase in headspace pressure caused by gas production by the proliferating organisms. It can take a considerable number of hours before the organisms cause a sufficient increase in headspace pressure to enable growth to be recognised. Although this tells the clinician that micro-organisms are growing within the bottle and therefore were present in the original sample, the identity of those micro-organisms is unknown at that

stage. With presently-available systems the best identification possible, in systems using selective media and/or using a change in carbon dioxide or oxygen content in the headspace gas, is whether the proliferating micro-organisms are aerobic or anaerobic. This information is of limited clinical benefit. Moreover, the original sample might contain more than one type of organisms. The culture must be subjected to further study, for example by plating and antibiotic susceptibility testing, before a specific pathogen can be identified. Thus, if the blood sample has been taken from a diseased patient, it is many hours before the possible cause of the patient's condition can be identified.

Therefore there is a need for a detection system which enables the presence of viable micro-organisms within the inoculated culture to be ascertained sooner. Furthermore, it would be extremely beneficial if the early detection of micro-organisms could be combined with early identification of their species or genus. A clinical assessment of the patient's condition could therefore be obtained much sooner.

GENERAL DESCRIPTION OF THE INVENTION

By the invention we have found that the application of so-called "electronic nose" technology can substantially reduce the time required to detect micro-organisms in blood culture, and provides the additional possibility that simultaneously with the detection of micro-organisms a (preliminary) identification of the type of micro-organism present in the culture can be provided. Clinically useful information can thereby be obtained from a blood sample much more rapidly.

The invention provides a method of detecting micro-organisms in a liquid culture medium inoculated with a

sample suspected of containing micro-organisms, involving:

incubating the inoculated liquid culture medium for a period of time sufficient to encourage micro-organism metabolism; and

detecting whether micro-organism metabolism has occurred by determining, in a gaseous atmosphere adjacent the liquid culture medium, the presence or concentration of a volatile compound which is generated, consumed or modified by metabolising micro-organisms.

Preferably, determination of the presence or concentration of the volatile compound is conducted periodically during the incubation period, and an indication that micro-organism metabolism has occurred is given when a pre-set level of the volatile compound is detected.

Preferably, the determination of the presence and/or concentrations of a plurality of volatile compound provides a 'finger-print' indicative of the presence of a particular genus or species of micro-organism. Ideally, the 'finger-print' is determined by means of an 'electronic nose'.

The invention is particularly applicable to blood culture.

Ideally, the method of the invention is conducted using conventional culture bottles, and the gaseous atmosphere within such bottles comprises the headspace gas. Preferably, the headspace gas pressure is also monitored to provide a further indication of micro-organism presence.

The invention also provides apparatus for detecting the presence of micro-organisms in a sample, comprising:

a container in which a liquid culture medium inoculated with the sample can be incubated; and

means for detecting within a gaseous atmosphere adjacent to the liquid culture medium while the medium is being incubated, the presence or concentration of one or more volatile compounds which are generated, consumed or modified by metabolising micro-organisms. Optionally, the apparatus additionally comprises means to indicate when the presence or concentration of a volatile compound being detected has attained or fallen to a pre-set level.

An important embodiment of the invention is a blood culture facility comprising:

an incubation chamber for containing a plurality of a blood culture bottles;

means for individually sampling continuously or intermittently the headspace gas within culture bottles placed within the chamber;

'electronic nose' means for determining a volatile compound 'finger-print' in each sampled headspace gas;

electronic means associated with the 'electronic nose' means, programmed to identify a volatile compound 'finger-print' change indicative of the metabolism of micro-organisms within an individual culture bottle, and preferably also to derive from the changed volatile compound 'finger-print' the identity of a genus or species of micro-organisms present within the individual culture bottle; and, associated with the electronic means,

visual display means and/or print-out means to reveal the presence and/or identity of micro-organisms within one or more of the incubating culture bottles. Optionally, the facility additionally comprises means for detecting within any one of the individual incubating culture bottles a change in headspace gas pressure indicative of the presence

of micro-organisms.

The skilled reader will appreciate that in the context of the invention the expression "volatile compound" is being used herein to denote a compound that is produced only in trace amounts by a metabolising micro-organism. A volatile compound is therefore quite different from the gaseous materials, especially oxygen and carbon-dioxide, which may be consumed or generated in abundance by proliferating micro-organisms and which therefore lead to gross effects such as detectable pressure changes.

Appropriate "electronic nose" technology is described, for example, in 'Sensor arrays using conducting polymers for electronic nose', Chapter 15 in "Sensors and Sensory System for an Electronic Nose", Eds. Gardner, JW and Bartlett, PN, NATO ASI Series, Series E, Applied Sciences - 212, 237, (1992); and Hodgins, D, 'The Electronic Nose - A new concept in comparative analysis', Brewer's Guardian, 24, July (1993).

Identification of the presence of micro-organisms in accordance with the invention can be determined by observing the presence and/or concentration of one or more specific volatile compounds in the gaseous atmosphere. From the scientific literature it is already known that various species of micro-organisms generate characteristic volatile compounds. By tuning the system to the identification of one or more pre-determined volatile compounds it can be rendered species/genus specific. Examples of suitable volatile compounds and species/genus with which they are already associated are given below in Table 1.

However, as is the case with the presently available "electronic nose" technology, the invention can make use of a multiplicity of sensors, each of which is based on a

different reactive polymer or other material, which together provide a complex and unique response to an "odour profile" without necessarily identifying specific volatile components of that odour. By comparing this complex response from the sensors with a standardized response from known micro-organisms species cultured under comparable conditions, a positive identification of the metabolising micro-organisms can be derived.

By practice of the invention we have found that it is possible to recognise the presence of microorganisms within a blood culture system within merely 4-6 hours in case of microorganisms of the species Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Candida albicans. This is a considerable improvement on the normal response time within conventional blood culture systems wherein observation of the growth of these species is not normally possible until after 18-50 hours of incubation. A distinctive "finger-print" associated with these species can be obtained concurrently using the invention. Conventional identification procedures which must follow the incubation stage normally take an additional 24-48 hours.

The gaseous headspace atmosphere within a culture bottle is already saturated with volatile components from the culture medium itself. At least during the initial stages of micro-organism proliferation the amounts of distinctive volatile compounds associated with those organisms will be very small and one would expect their presence to be masked by the volatiles from the medium. It is therefore most surprising that a system based on volatile compound detection can provide such an early indication of micro-organism presence and, additionally, provide worthwhile information on species or genus identity.

By way of example only, a blood culture facility in

accordance with the invention will now be described with reference to the accompanying drawings, of which:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the general layout of a blood culture facility for handling a plurality of conventional blood culture bottles;

Figure 2 illustrates in cross-section an individual blood culture bottle with means for linking the bottle to an 'electronic nose'; and

Figures 3 and 3a depict a "cluster map" showing different multi-sensor responses to the volatile compound fingerprints of a selection of common bacterial species after several hours incubation.

DETAILED DESCRIPTION OF THE INVENTION

Referring to Figure 1, the apparatus comprises an incubation chamber 110 having a front loading facility 111. The incubation chamber is shown in partially cut-away form to reveal the interior. The chamber 110 contains a tray 112 having a plurality of individual recesses 113 each of which can contain a conventional blood culture bottle 114. An array of bottles is standing within tray 112. For easy access to the bottles, the tray can be slid or otherwise moved in and out of the chamber via the front loading facility, but this aspect is not critical to the invention.

Each culture bottle 114 has an overcap 115 which is linked via a flexible tube 116 to a central location in the roof 117 of the chamber. At this location an external "junction box" 118 provides means for connecting the internal tubes 116 to a single external tube 119 which leads to an "electronic nose" facility 120. Linked to the electronic

nose is a micro-processor/VDU 121.

Incubation chamber 110 is with provided heating means (not shown) and temperature-regulating means (also not shown). Ideally the incubation chamber is also provided with means for magnetically stirring the contents of each culture bottle, as described in WO 94/02238.

Referring to Figure 2, each individual culture bottle 114 is of the conventional upright cylindrical glass construction with a cylindrical neck 200 of narrower diameter than the body of the bottle. The top of the bottle is sealed by a conventional rubber septum 201. An overcap 115, for example moulded from plastics material, is secured on the top of the bottle, extending over the entire septum. For example, this overcap can be a "push fit" onto the top of the bottle or can be clipped thereon by resilience in the moulding. Overcap 115 is provided with a centrally-disposed downwardly-projecting hollow needle 202 which pierces the septum when the overcap is applied to the bottle. The needle extends downwardly into the gaseous headspace 203 within the bottle, but does not reach the surface 204 of liquid growth medium 205 in the bottle. Within the overcap, at the top of the needle, is a bacterial filter 206 which readily permits passage of gaseous and volatile components but prevents micro-organism cells from escaping from the bottle via the needle. A short, centrally-disposed tubular extension 207 projects upwardly from the top of the overcap to provide an outlet for gaseous/volatile material through the needle. This tubular extension provides an application point for an external flexible tube 116 which can lead the gaseous/volatile material away to an analytical facility such as "electronic nose". The connection 208 between the tubular extension 207 and the external tube 115 can be a simple "push fit" as depicted, or can be provided with more positive locating means such as cooperating screw threads.

Resting on the bottom of the bottle is a magnetic "flea" 209 which can be driven by external electromagnetic means (not shown) to agitate the contents of the bottle during incubation.

Depending for example on the number of sensing heads within the electronic nose, this facility can either monitor each culture bottle continuously during the incubation or can monitor each bottle intermittently for example, every 30 minutes. If desired one group of sensors can monitor each bottle in turn.

In operation, each individual culture bottle is injected with a blood sample. An overcap is placed on each bottle such that the hollow needle pierces the septum. The outlet from the overcap is connected to a tube within the incubator while the bottle is being loaded into the incubator. This operation is performed in accordance with an established laboratory procedure, to ensure that the identity of each bottle is carried through into the sensing facility. At a rudimentary level this can be achieved by each bottle having an identifiable code which is associated with a particular tube within the incubator. The operator can input this code to the PC manually. Alternatively an automated reading system, such as a barcode, can be used.

While the bottles are being incubated their headspace gases can be monitored for the presence of or changes in the concentration of volatile compounds associated with micro-organism metabolism. Information in this regard is derived by the electronic nose and relayed to the PC. The PC has been programmed to evaluate this information and to derive from it an indication that micro-organism metabolism is actually occurring in a particular culture bottle. In addition, by comparing the "finger-print" derived by the electronic nose from the sampling from the headspace gas in an individual bottle with known "finger-prints" from

commonly-occurring micro-organisms, an indication is given of the likely species or genus which is proliferating within the bottle. The operator can be alerted to the fact that some bottles are proving "positive", for example by information appearing on the VDU screen. The screen can also convey more information, for example the likely identity of the micro-organisms.

By means of such a facility a clinically useful assessment of blood samples can be provided rapidly.

The overcap, as described above, which during use is likely to become contaminated on its inner surface by material from the sample, can be manufactured cheaply as a disposable item. The microbial filter prevents any such contamination reaching the "electronic nose" equipment during careful use of the facility.

EXAMPLES

The following experiments show the advantageous application of "electronic nose" technology in the field of blood culture.

Methodology

A blood-culture medium, in a conventional septum-sealed culture bottle, was inoculated with a target dilution of one of a range of bacterial suspensions. Each inoculated bottle was fitted with a commercially-available "SIGNAL" (TM) pressure-based bacterial growth detection device, as described in EP-A-124193. This device has a needle extending downwardly into the culture bottle and entering the liquid medium, permitting the medium to be expressed upwardly into a chamber above the needle to indicate visibly an increase in headspace gas pressure within the bottle. The chamber is vented to the atmosphere, allowing

in this instance the atmosphere in contact with the medium to be readily accessible to an electronic nose facility. An identical control was inoculated with sterile saline solution. A typical blood culture bottle as supplied commercially contains about 80 ml of culture medium. A typical "all-purpose" aqueous formulation, as used in this experiment, is (in gm per litre):

Phosphate buffer	0.288
Tryptone Soya Broth	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Meat extract	5.0
Glucose	1.0
Sodium chloride	8.0
L-Arginine	1.0
Sodium Pyruvate	1.0
Menadione	0.005
Gelatin	1.0
Sodium thioglycollate	0.5
Cysteine HCl	0.4
Sodium bicarbonate	0.4
Ammonium chloride	0.008
Dithiothreitol	0.2
Adenine sulphate	0.01
Sodium succinate	0.01
Potassium nitrate	2.0
Magnesium sulphate	0.008
sulphonate	0.3
pH	7.0

The bottles were incubated at 37°C and monitored by a "BLOODHOUND" (TM) "electronic nose" after 4 hours incubation. The system used an array of 16 different sensors, based on reactive polymers. The "electronic nose" system was recorded as positive when significant response differences where apparent when compared to the control.

The conventional system was recorded as a positive when the liquid medium was visibly displaced into the upper chamber of the device, as described in EP-A-124193.

Some typical results are shown in Table 2, where the electronic nose indicated positive evidence of microbial presence after 4 to 6 hours, compared to a minimum of 18 hours for the conventional system.

Figure 3 shows other results represented as "cluster maps" of the complex sensor response to various organisms, again after only 4 to 6 hours incubation as described above. In Figure 3 the complex sensor response had been represented originally as an odourgram, ie. a polar plot representation f the responses of individual numbered sensor electrodes (1 to 16) to odour molecules (see Figure 3a for a typical example). The clusters depicted in Figure 3 are derived from such polar plots, the two principal dimensions being given in arbitrary units. In Figure 3, the clusters depicted show the complex response to the following organisms:

A	Control	After 6 hours
B	Staph. aureus	After 6 hours
C	Pseudomonas	After 6 hours
D	Candida	After 6 hours
E	E.coli	After 4 hours

It will be appreciated that a different selection of reactive sensors may yield a different response profile, and lead to a "positive" signal after a longer or shorter incubation period. Nevertheless, the principle will be the same. Within a range of available sensors, a selection can be made to achieve a rapid and distinctive "positive" response.

TABLE 1 (Part 1)

Volatil compounds associated with microbial metabolism

Volatile Compound	Pseudo-monas fluor-escens	Pseudo-monas putida	Pseudo-monas fragi	Altero-monas putre-faciens	Serratia lique-faciens	Brocho-thrix thermo-sphacta
2-6 Dithianonane			X			
Methyl proppyl-trisulphide				X		
S-N Compound M 163					X	X
S-N Compound M 189					X	X
1-Undecanol	X					
2-Propanol		X				
2-Butanol		X				
2-Octanol		X				
1,4 Butanediol			X			
2-Methyl-propanal						X
2-Methyl-butanal	X		X			X
3-Methyl-butanal			X	X	X	X

TABLE 1 (Part 2)

Volatile Compound	Pseudo-monas fluor-escens	Pseudo-monas putida	Pseudo-monas fragi	Altero-monas putre-faciens	Serratia lique-faciens	Brocho-thrix thermo-sphacta
2-Octanone		X				
2-Decanone		X				
3-Hexanone		X				
4-Methyl-2-pentanone		X				
4-Methyl-2-heptanone		X				
5-Hepten-2-one		X				
7-Octen-2-one		X				
Acetoin						X
Propanoic acid octylester			X			
Butanoic acid methylester			X			
Butanoic acid propylester			X			
Pentanoic acid ethylester			X			

TABLE 1 (Part 3)

Volatile Compound	Pseudo-monas fluor-escens	Pseudo-monas putida	Pseudo-monas fragi	Altero-monas putre-faciens	Serratia lique-faciens	Brocho-thrix thermo-sphacta
Decanoic acid ethylester			X			
2-Methyl butanoic acid propylester			X			
4-Methyl pentanoic acid methylester			X			
4-Hydroxy-3-pentenoic acid methylester						X
2-Methyl propanoic acid						X
2-Methyl butanoic acid						X
3-Methyl butanoic acid.						X

TABLE 2

Microorganism	Inoculum level (CFU's per ml).	Conventional detection time (hours)	Detection time by "electronic nose (hours)
Escherichia coli	50	18	4
Pseudomonas aeruginosa	75	36	6
Staphyloccus aureus	60	36	6
Candida albicans	8	50	6

CLAIMS

1. A method of detecting micro-organisms in a liquid culture medium inoculated with a sample suspected of containing micro-organisms, involving:

incubating the inoculated liquid culture medium for a period of time sufficient to encourage micro-organism metabolism; and

detecting whether micro-organism metabolism has occurred by determining a gaseous atmosphere adjacent the liquid culture medium, the presence or concentration of a volatile compound which is generated, consumed or modified by metabolising micro-organisms.

2. A method according to claim 1, involving the determination of the presence and/or concentration of a plurality of volatile compounds to provide a "finger-print" indicative of the presence of a particular genus or species of micro-organism.

3. A method according to claim 2, wherein the "finger-print" is determined by means of an "electronic nose".

4. A blood culture method according to any one of the preceding claims.

5. A method according to any one of the preceding claims, wherein the incubation is conducted within a culture bottle, and the gaseous atmosphere comprises the headspace gas.

6. A method according to claim 5, wherein the headspace gas pressure is also monitored to provide a further indication of micro-organism presence.

7. Apparatus for detecting the presence of micro-organisms in a sample, comprising:

a container in which a liquid culture medium inoculated with the sample can be incubated; and

means for detecting within a gaseous atmosphere adjacent to the liquid culture medium while the medium is being incubated, the presence or concentration of one or more volatile compounds which are generated, consumed or modified by metabolising micro-organisms.

8. Apparatus according to claim 7, additionally comprising means to indicate when the presence or concentration of a volatile compound being detected has attained or fallen to a pre-set level.

9. A blood culture facility comprising:

an incubation chamber for containing a plurality of blood culture bottles;

means for individually sampling continuously or intermittently the headspace gas within culture bottles placed within the chamber;

"electronic nose" means for determine a volatile compound "finger-print" in each sampled headspace gas;

electronic means associated with the "electronic nose" means, programmed to identify a volatile compound "finger-print" change indicative of the metabolism of micro-organisms within an individual culture bottle, and preferably also to derive from the changed volatile compound "finger-print" the identity of a genus or species of micro-organism present the individual culture bottle; and, associated with the electronic means,

visual display means and/or print-out means to reveal the presence and/or identity of micro-organisms within one or more of the incubating culture bottles.

10. A blood culture facility according to claim 9, additionally comprising means for detecting within any one of the individual incubating culture bottles a change in headspace gas pressure indicative of the presence of organisms.

Fig.1.

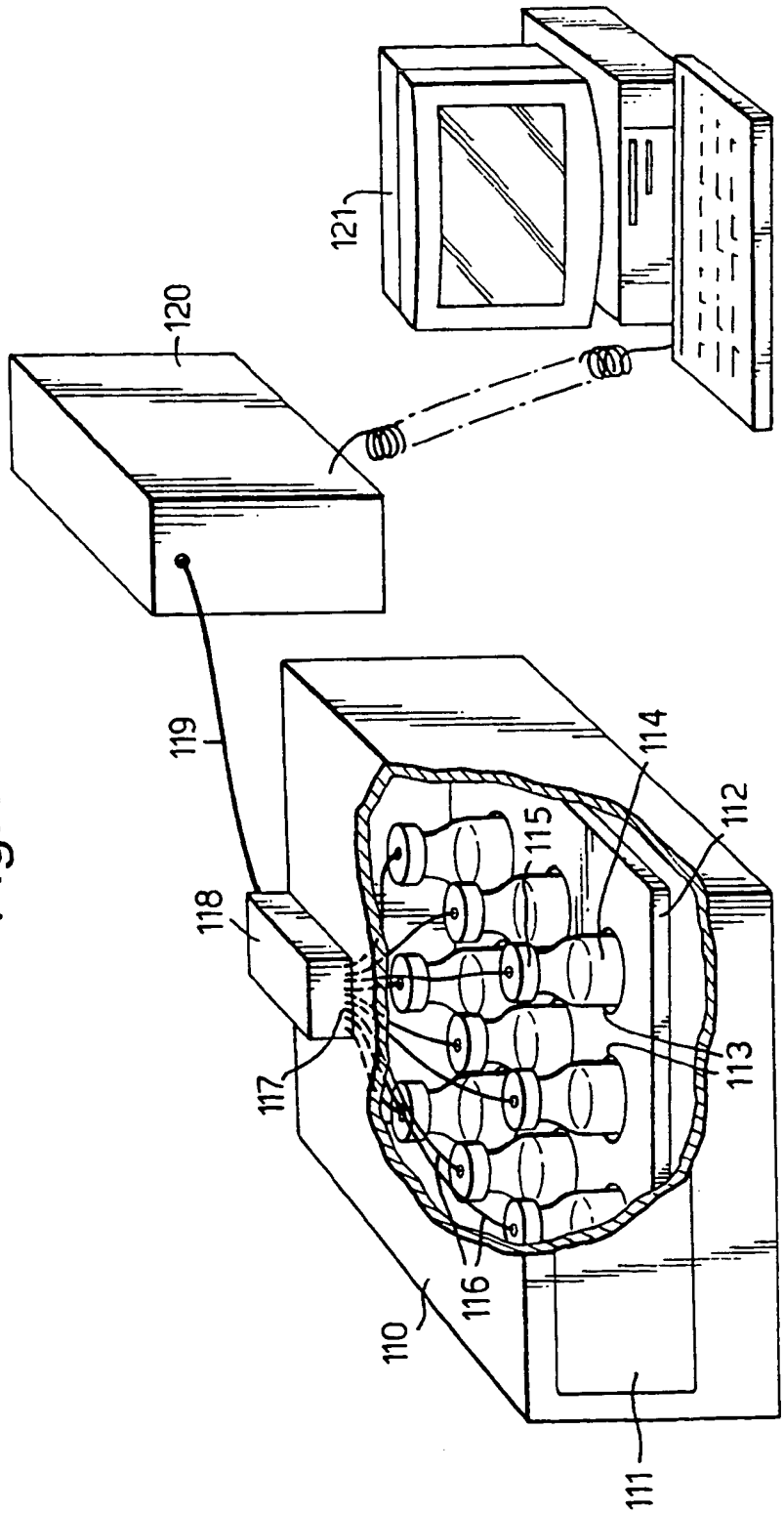
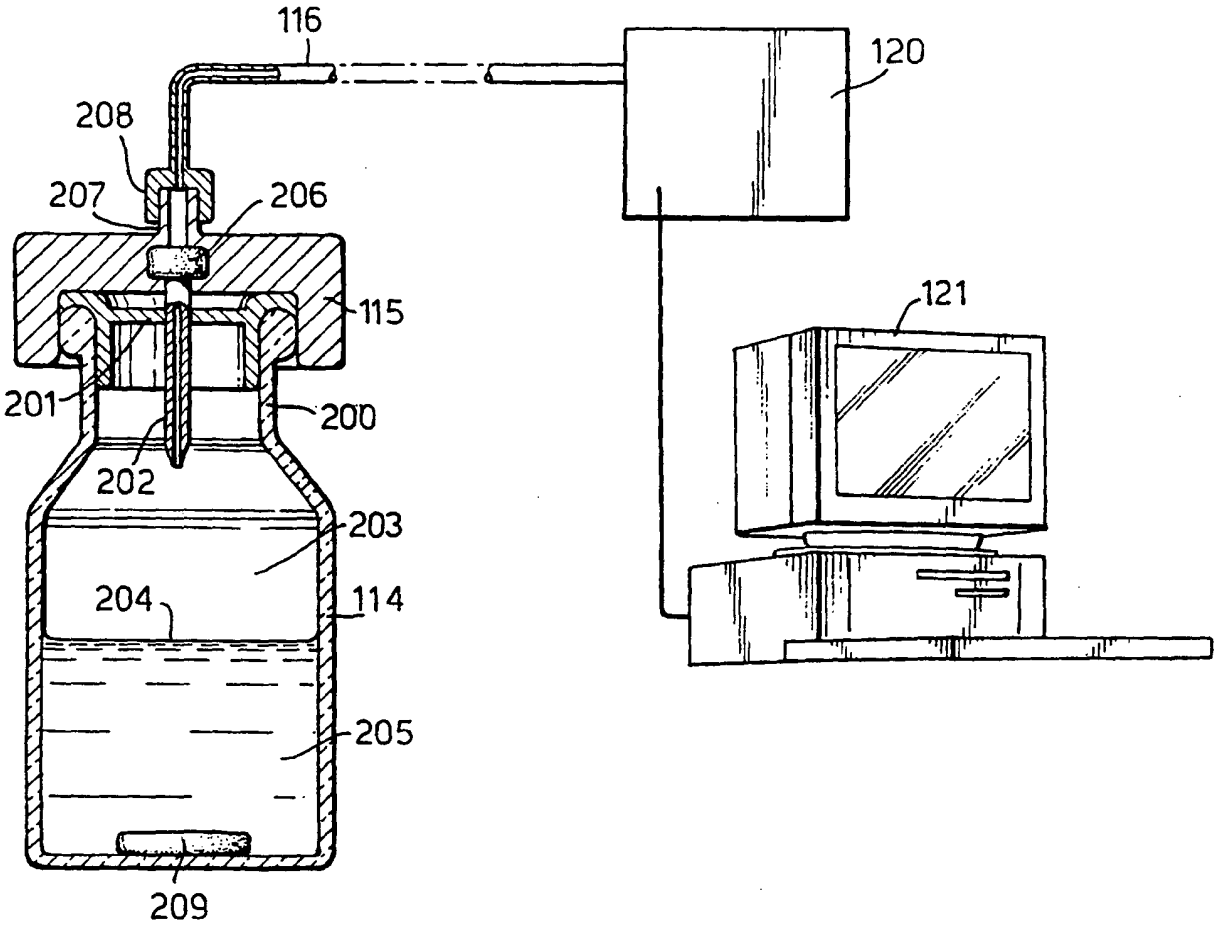


Fig.2.



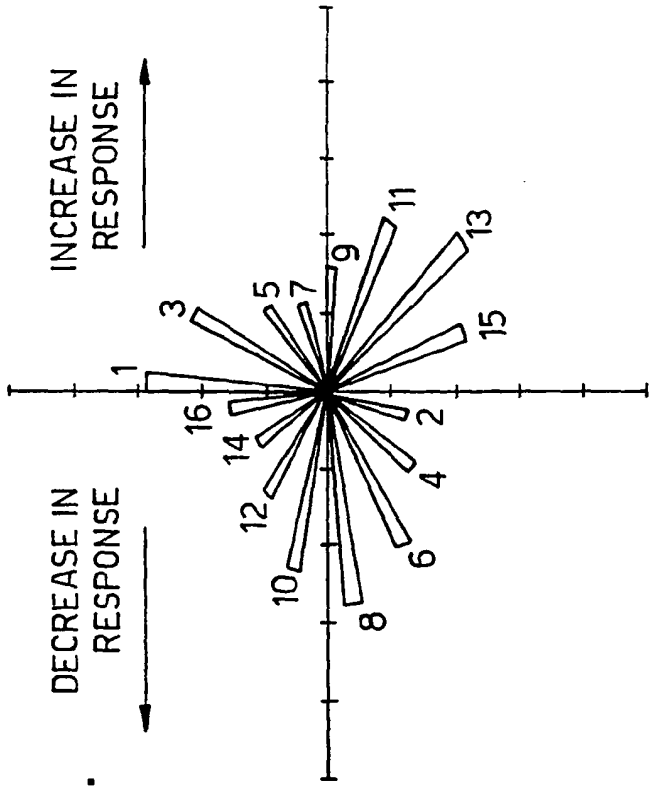

INCREASE IN RESPONSE
DECREASE IN RESPONSE
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Fig.3a.

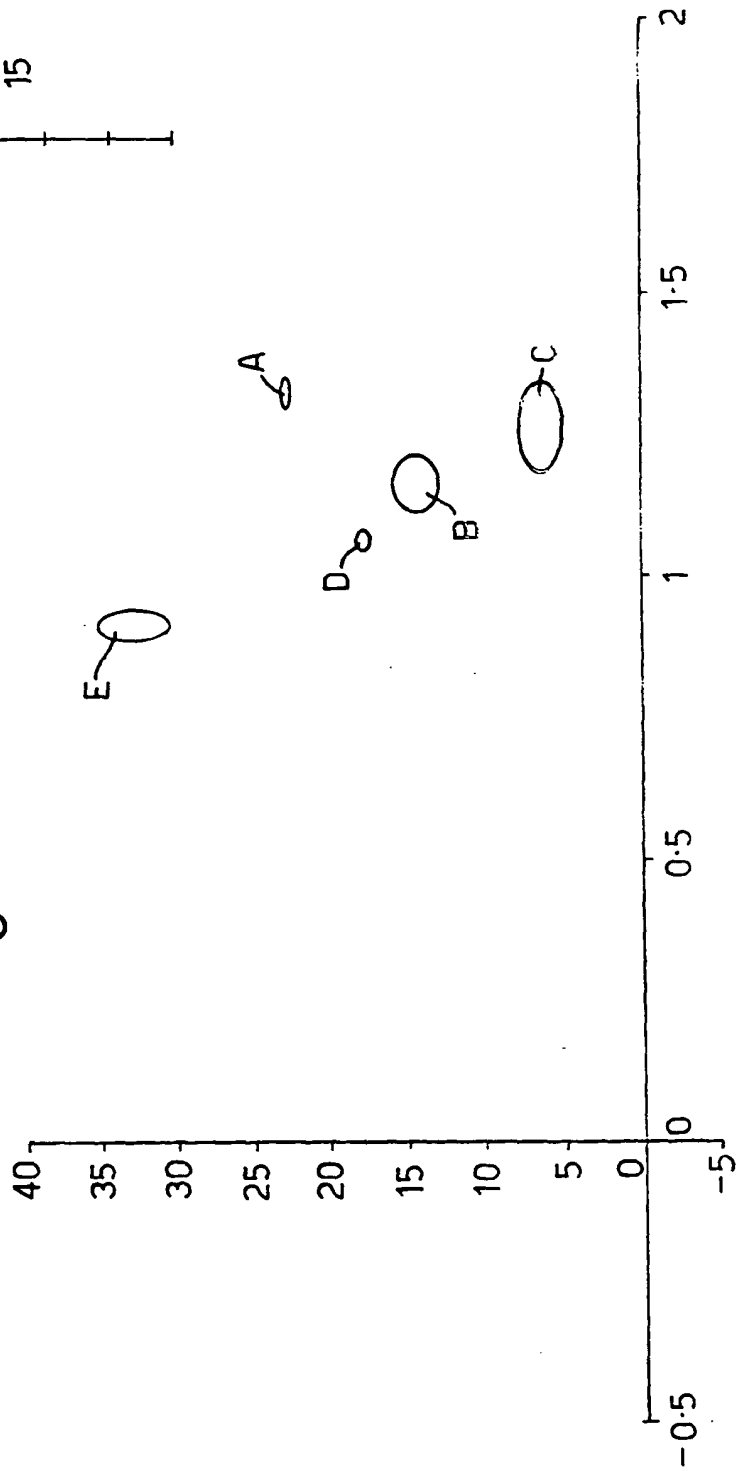
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Fig.3.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 96/03604

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/04 G01N33/497

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q G01N C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 04705 (THE MINISTER OF AGRICULTURE FISHERIES AND FOOD) 3 March 1994 see the whole document ---	1,3,5,7
X	WO,A,90 13663 (AVL AG) 15 November 1990 see the whole document ---	1,2,4-9
X	EP,A,0 158 497 (BECTON DICKINSON AND COMPANY) 16 October 1985 see the whole document ---	1,7,9
X	EP,A,0 124 193 (OXOID LIMITED) 7 November 1984 cited in the application see the whole document --- -/--	1,4-6

[X] Further documents are listed in the continuation of box C.

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9 December 1996	06. 01. 97

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Form PCT/ISA/210 (second sheet) (July 1992)

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PCT/EP 96/03604

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHINESE JOURNAL OF MICROBIOLOGY AND IMMUNOLOGY, vol. 19, no. 1, 1986, TAIPEI TAIWAN REPUBLIC OF CHINA, pages 18-26, XP000196638 S-W. HO: "Head-space gas-liquid chromatographic analysis for presumptive identification of bacteria in blood cultures" see the whole document	1,2,4-7
A	WO,A,95 08113 (ALPHA M.O.S.) 23 March 1995 see the whole document	1,3,7,8
A	SENSORS AND ACTUATORS B, vol. b18, no. 1/3, March 1994, LAUSANNE CH, pages 282-290, XP000450920 P.-M SCHWEIZER-BERBERICH ET AL.: "Characterisation of food freshness with sensor arrays" see the whole document	1-3,7,8
A	J.W. GARDNER AND P.N. BARTLETT (EDS.): "NATO ASI Series E: Sensors and sensory systems for an electronic nose" 1992 , KLUWER ACADEMIC PUBLISHERS , AMSTERDAM NL XP000196647 cited in the application see page 237 - page 256	1,3,9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/03604

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 3,9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Lack of technical disclosure.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int onal Application No
PCT/EP 96/03604

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9404705	03-03-94	AU-A-	4967493	15-03-94
		EP-A-	0656066	07-06-95
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WO-A-9508113	23-03-95	FR-A-	2710153	24-03-95
		EP-A-	0719411	03-07-96